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08/552,839
attachment to
Paper #14

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FILE 'USPAT' ENTERED AT 12:40:59 ON 09 MAR 1999

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  *                W E L C O M E   T O   T H E                  *
  *          U. S.   P A T E N T   T E X T   F I L E            *
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=> s adenovir? and vector?

          4162 ADENOVIR?
         76009 VECTOR?
L1        3331 ADENOVIR? AND VECTOR?

=> s l1 and early region 4

        103206 EARLY
        438233 REGION
       2364327 4
             5 EARLY REGION 4
                 (EARLY(W)REGION(W)4)
L2           5 L1 AND EARLY REGION 4

=> d l2,1-5,cit,ab


1.  5,851,806, Dec. 22, 1998, Complementary adenoviral systems and
cell lines; Imre Kovesdi, et al., 435/91.41, 320.1, 325, 366; 536/24.2
[IMAGE AVAILABLE]

US PAT NO:      5,851,806 [IMAGE AVAILABLE]            L2: 1 of 5

ABSTRACT:
The present invention provides multiply replication deficient
adenoviral vectors having a spacer in at least one replication
deficient adenoviral region, as well as complementing cell lines
therefor. Also provided are means of constructing the multiply
replication deficient adenoviral vectors and methods of use
thereof, e.g., in gene therapy.

2.  5,837,511, Nov. 17, 1998, Non-group C adenoviral vectors;
Erik S. Falck-Pedersen, et al., 435/6, 235.1, 320.1, 325, 456; 514/44
[IMAGE AVAILABLE]

US PAT NO:      5,837,511 [IMAGE AVAILABLE]            L2: 2 of 5

ABSTRACT:
The present invention provides replication-deficient non-group C
adenoviral vectors. Also provided is a therapeutic method,
particularly relating to gene therapy, vaccination, and the like,
involving the use of such vectors incorporating a foreign nucleic
acid.

3.  5,670,488, Sep. 23, 1997, Adenovirus vector for gene therapy;
Richard J. Gregory, et al., 514/44; 424/93.2; 435/320.1 [IMAGE AVAILABLE]

US PAT NO:      5,670,488 [IMAGE AVAILABLE]            L2: 3 of 5

ABSTRACT:
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Gene Therapy **vectors**, which are especially useful for cystic fibrosis, and methods using the **vectors** are disclos

4. 5,639,661, Jun. 17, 1997, Genes and proteins for treating cystic fibrosis; Michael J. Welsh, et al., 435/252.3, 320.1; 536/23.5, 24.3 [IMAGE AVAILABLE]

US PAT NO: 5,639,661 [IMAGE AVAILABLE] L2: 4 of 5

ABSTRACT:
Disclosed are genes encoding novel CF monomer proteins which have cystic fibrosis transmembrane conductance regulator (CFTR) protein function.

5. 5,616,326, Apr. 1, 1997, Recombinant canine **adenovirus** 2 (CAV-2); Norman Spibey, 424/199.1, 205.1, 207.1, 208.1, 224.1, 233.1, 818, 819; 435/235.1, 252.3, 320.1 [IMAGE AVAILABLE]

US PAT NO: 5,616,326 [IMAGE AVAILABLE] L2: 5 of 5

ABSTRACT:
A non-essential region in strains of live non-pathogenic immunogenic canine **adenovirus** is described. The insertion of genes from pathogenic carnivora viruses into this region, with suitable expression control systems, without prejudicing the stable reproducibility of the **adenovirus vector** is described. Such recombinant canine **adenoviruses** modified to contain a gene coding for an antigen or immunogenic agent, in association with an effective promoter for the gene, are described.

=> d 1,3,fro

US PAT NO: 5,851,806 [IMAGE AVAILABLE] L2: 1 of 5
DATE ISSUED: Dec. 22, 1998
TITLE: Complementary **adenoviral** systems and cell lines
INVENTOR: Imre Kovesdi, Rockville, MD
Douglas E. Brough, Olney, MD
Duncan L. McVey, Derwood, MD
Joseph T. Bruder, Gaithersburg, MD
Alena Lizonova, Rockville, MD
ASSIGNEE: GenVec, Inc., Rockville, MD (U.S. corp.)
APPL-NO: 08/572,126
DATE FILED: Dec. 14, 1995
REL-US-DATA: Continuation-in-part of Ser. No. 258,416, Jun. 10, 1994.
INT-CL: [6] C12P 19/34; C12N 15/11; C12N 5/16; C12N 5/22
US-CL-ISSUED: 435/91.41, 320.1, 325, 366; 536/24.2
US-CL-CURRENT: 435/91.41, 320.1, 325, 366; 536/24.2
SEARCH-FLD: 435/69.1, 91.1, 235.1, 246.1, 320.1, 91.41, 172.3, 325, 366; 536/23.1, 24.1, 24.2
REF-CITED:

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| 2 707 664 | 1/1995 | France |
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| WO 94/11506 | 5/1994 | World Intellectual Property Organization |
| WO 94/12649 | 9/1994 | World Intellectual Property |

| | | |
|---|---|---|
| PCT/US95/14793 | 11/[redacted] | Organization<br>World Intellectual Property<br>Organization<br>424/78.08 |
| WO 94/28938 | 12/1994 | World Intellectual Property<br>Organization |
| WO 95/00655 | 1/1995 | World Intellectual Property<br>Organization |

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ART-UNIT: 185
PRIM-EXMR: David Guzo
ASST-EXMR: William Sandals
LEGAL-REP: Leydig, Voit & Mayer, Ltd.

ABSTRACT:
The present invention provides multiply replication deficient **adenoviral vectors** having a spacer in at least one replication deficient **adenoviral** region, as well as complementing cell lines therefor. Also provided are means of constructing the multiply replication deficient **adenoviral vectors** and methods of use thereof, e.g., in gene therapy.
63 Claims, 21 Drawing Figures

US PAT NO: 5,670,488 [IMAGE AVAILABLE] L2: 3 of 5
DATE ISSUED: Sep. 23, 1997
TITLE: **Adenovirus vector** for gene therapy
INVENTOR: Richard J. Gregory, Carlsbad, CA
Donna Armentano, Watertown, MA
Larry A. Couture, Framingham, MA
Alan E. Smith, Wellesley, MA
ASSIGNEE: Genzyme Corporation, Framingham, MA (U.S. corp.)
APPL-NO: 08/136,742
DATE FILED: Oct. 13, 1993
REL-US-DATA: Continuation-in-part of Ser. No. 985,478, Dec. 3, 1992, abandoned.
INT-CL: [6] A61K 48/00; C12N 15/00
US-CL-ISSUED: 514/44; 424/93.2; 435/320.1; 935/62
US-CL-CURRENT: 514/44; 424/93.2; 435/320.1
SEARCH-FLD: 435/320.1; 514/44; 424/93.2; 935/62
REF-CITED:
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4,920,209 4/1990 Davis

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Berker (1988) Biotechniques 6, 616-627.
ART-UNIT: 189
PRIM-EXMR: Deborah Crouch
LEGAL-REP: Brumbaugh, Graves Donohue & Raymond

ABSTRACT:
Gene Therapy **vectors**, which are especially useful for cystic fibrosis, and methods for using the **vectors** are disclosed.
19 Claims, 67 Drawing Figures

=> d 1,3,clms

US PAT NO: 5,851,806 [IMAGE AVAILABLE] L2: 1 of 5

CLAIMS:

CLMS(1)

What is claimed is:

1. An **adenoviral vector** comprising (a) an **adenoviral** genome deficient in the E4 region of the **adenoviral** genome,, (b) a spacer of at least 15 base pairs in the E4 region of the **adenoviral** genome, and (c) a passenger gene, wherein said spacer comprises a sequence that is non-native to the E4 region of the **adenoviral** genome, and wherein the viral production level of said **adenoviral vector** is greater than the viral production level of said **adenoviral vector** without said spacer.

CLMS(2)

2. The **adenoviral vector** of claim 1, wherein the viral production level of said **adenoviral vector** without said spacer and without said passenger gene is lower than the viral production level of said **adenoviral vector** without said spacer, said passenger gene, and said deficiency in the E4 region of the **adenoviral** genome.

CLMS(3)

3. A host cell comprising the **adenoviral vector** of claim 1.

CLMS(4)

4. A composition comprising the **adenoviral vector** of claim 1,

and a carrier therefor.

CLMS(5)

5. A replication competent **adenovirus**-free stock of the **adenoviral vector** of claim 1, wherein said spacer comprises said passenger gene, wherein said **adenoviral vector** is prepared in a cell line which will support the growth of said **adenoviral vector**, and wherein the genome of said cell line is free of overlapping sequences with said **adenoviral vector** such that said cell genome will not mediate a recombination event which would produce a replication competent **adenoviral vector**.

CLMS(6)

6. A method of genetically modifying a cell in vitro, which comprises contacting said cell with the **adenoviral vector** of claim 1.

CLMS(7)

7. The **adenoviral vector** of claim 1, wherein said **adenoviral** genome is deficient in one or more essential gene functions of the E4 region of the **adenoviral** genome.

CLMS(8)

8. The **adenoviral vector** of claim 7, wherein said **adenoviral** genome is deficient in one or more essential gene functions of another region of the **adenoviral** genome.

CLMS(9)

9. The **adenoviral vector** of claim 8, wherein said another region is the E1 region of the **adenoviral** genome.

CLMS(10)

10. The **adenoviral vector** of claim 1, wherein said **adenoviral** genome is deleted of the E4 region of the **adenoviral** genome.

CLMS(11)

11. The **adenoviral vector** of claim 10, wherein said **adenoviral** genome is deficient in one or more other essential gene functions of another region of the **adenoviral** genome.

CLMS(12)

12. The **adenoviral vector** of claim 1, wherein said **adenoviral** genome is deleted of the E4 region of the **adenoviral** genome except the E4 promoter and the E4 polyadenylation sequence.

CLMS(13)

13. The **adenoviral vector** of claim 12, wherein said **adenoviral** genome is deficient in one or more other essential gene functions of another region of the **adenoviral** genome.

CLMS(14)

14. The **adenoviral vector** of claim 1, wherein said **adenoviral** genome is deleted of the E4 region of the **adenoviral** genome except the E4 polyadenylation sequence.

CLMS(15)

15. The **adenoviral vector** of claim 14, wherein said **adenoviral** genome is deficient in one or more essential gene functions of another region of the **adenoviral** genome.

CLMS(16)

16. The **adenoviral vector** of claim 1, wherein said **adenoviral** genome is further deficient in the E2A region of the **adenoviral** genome.

CLMS(17)

17. The **adenoviral vector** of claim 16, wherein said E2A region comprises a portion of the **adenoviral** genome which encodes amino acids of the Nt domain of the DBP sufficient to allow for viral production, and wherein said E2A region is deficient in the portion of the **adenoviral** genome which encodes amino acid sequences of the Ct domain that are necessary for DNA binding.

CLMS(18)

18. The **adenoviral vector** of claim 1, wherein said spacer is about 100 to about 12,000 base pairs.

CLMS(19)

19. A method of genetically modifying a cell in vitro, which comprises contacting said cell with the **adenoviral vector** of claim 18.

CLMS(20)

20. The **adenoviral vector** of claim 18, wherein said spacer is about 500 to about 8,000 base pairs.

CLMS(21)

21. The **adenoviral vector** of claim 20, wherein said spacer is about 1,500 to about 6,000 base pairs.

CLMS(22)

22. The **adenoviral vector** of claim 1, wherein said spacer comprises a polyadenylation sequence other than an E4 **adenoviral** polyadenylation sequence.

CLMS(23)

23. The **adenoviral vector** of claim 22, wherein said spacer comprises an SV40 polyadenylation sequence.

CLMS(24)

24. A method of genetically modifying a cell in vitro, which comprises-contacting said cell with the **adenoviral vector** of claim 22.

CLMS(25)

25. A replication competent **adenovirus**-free stock of the **adenoviral vector** of any of claims 1, 2 and 7-17, wherein said spacer comprises a polyadenylation sequence other than an E4 **adenoviral** polyadenylation sequence.

CLMS(26)

26. The replication competent **adenovirus**-free stock of claim 25, wherein said spacer comprises an SV40 polyadenylation sequence.

CLMS(27)

27. A replication competent **adenovirus**-free stock of the **adenoviral vector** of claim 1, wherein said spacer comprises a polyadenylation sequence other than an E4 **adenoviral** polyadenylation sequence, wherein said **adenoviral vector** is prepared in a cell line which will support the growth of said **adenoviral vector**, and wherein the genome of said cell line is free of overlapping sequences with said **adenoviral vector** such that said cell genome will not mediate a recombination event which would produce a replication competent **adenoviral vector**.

CLMS(28)

28. The replication competent **adenovirus**-free stock of claim 27, wherein said spacer comprises an SV40 polyadenylation sequence.

CLMS(29)

29. A replication competent **adenovirus**-free stock of the **adenoviral vector** of any of claims 1, 2 and 7-17, wherein said spacer comprises a polyadenylation sequence other than an E4 **adenoviral** polyadenylation sequence, wherein said **adenoviral vector** is prepared in a cell line which will support the growth of said **adenoviral vector**.

CLMS(30)

30. The replication competent **adenovirus**-free stock of claim 29, wherein said spacer comprises an SV40 polyadenylation sequence.

CLMS(31)

31. A replication competent **adenovirus**-free stock of the **adenoviral vector** of any of claims 1, 2 and 7-17, wherein said spacer comprises said passenger gene, wherein said **adenoviral vector** is prepared in a cell line which will support the growth of said **adenoviral vector**.

CLMS(32)

32. A method of increasing the propagation in a complementing cell line of an **adenoviral vector** comprising (a) an **adenoviral** genome deficient in the E4 region and (b) a passenger gene, which method comprises incorporating into the E4 region of the **adenoviral** genome of said **adenoviral vector** a spacer comprising at least about 15 base pairs, wherein said spacer comprises a sequence that is non-native to the E4 region of the **adenoviral** genome, thereby resulting in an increase in the viral production level of said **adenoviral vector**.

CLMS(33)

33. The method of claim 32, wherein said **adenoviral** genome is deficient in one or more essential gene functions of another region of the **adenoviral** genome.

CLMS(34)

34. The method of claim 32, wherein said **adenoviral** genome is deficient in one or more essential gene functions of the E4 region of the

**adenoviral** genome.

CLMS(35)

35. The method of claim 34, wherein said **adenoviral** genome is deficient in one or more essential gene functions of another region of the **adenoviral** genome.

CLMS(36)

36. The method of claim 34, wherein said **adenoviral** genome is further deficient in at least one or more essential gene functions of the E1 region of the **adenoviral** genome.

CLMS(37)

37. The method of claim 32, wherein said **adenoviral** genome is deleted of all the open reading frames of the E4 region of the **adenoviral** genome.

CLMS(38)

38. The method of claim 37, wherein said **adenoviral** genome is deficient in one or more essential gene functions of another region of the **adenoviral** genome.

CLMS(39)

39. The method of claim 32, wherein said **adenoviral** genome is deleted of the E4 region of the **adenoviral** genome except the E4 promoter and the E4 polyadenylation sequence.

CLMS(40)

40. The method of claim 39, wherein said **adenoviral** genome is deficient in one or more essential gene functions of another region of the **adenoviral** genome.

CLMS(41)

41. The method of claim 39, wherein said **adenoviral** genome is deleted of the E4 region of the **adenoviral** genome except the E4 polyadenylation sequence.

CLMS(42)

42. The method of claim 32, wherein said spacer is about 100 to about 12,000 base pairs.

CLMS(43)

43. The method of claim 42, wherein said spacer is about 500 to about 8,000 base pairs.

CLMS(44)

44. The method of claim 42, wherein said spacer is about 1,500 to about 6,000 base pairs.

CLMS(45)

45. The method of claim 32, wherein said spacer comprises a polyadenylation sequence other than an E4 **adenoviral** polyadenylation sequence.

CLMS(46)

46. The method of claim 45, wherein said spacer comprises an SV40 polyadenylation sequence.

CLMS(47)

47. An **adenoviral vector** comprising a portion of the E2A region of the **adenoviral** genome which encodes amino acids of the Nt domain of the DBP sufficient to allow for viral production, and wherein said E2A region is deficient in the portion of the **adenoviral** genome which encodes amino acid sequences of the Ct domain that are necessary for DNA bindings and further wherein the **adenoviral vector** comprises a passenger gene.

CLMS(48)

48. The **adenoviral vector** of claim 47, wherein said **adenoviral** genome is deficient in one or more essential gene functions of another region of the **adenoviral** genome.

CLMS(49)

49. An **adenoviral vector** comprising (a) an **adenoviral** genome deficient in the E4 region of the **adenoviral** genome, and (b) a spacer of at least 15 base pairs in the E4 region of the **adenoviral** genome, wherein said spacer comprises a passenger gene that is non-native to the E4 region of the **adenoviral** genome, and wherein the viral production level of said **adenoviral vector** is greater than the viral production level of said **adenoviral vector** without said spacer.

CLMS(50)

50. The **adenoviral vector** of claim 49, wherein said **adenoviral** genome is deficient in one or more essential gene functions of the E4 region of the **adenoviral** genome.

CLMS(51)

51. The **adenoviral vector** of claim 49, wherein the viral production level of said **adenoviral vector** without said spacer and without said passenger gene is lower than the viral production level of said **adenoviral vector** without said spacer, said passenger gene, and said deficiency in the E4 region of the **adenoviral** genome.

CLMS(52)

52. The **adenoviral vector** of claim 50, wherein said **adenoviral** genome is deficient in one or more essential gene functions of another region of the **adenoviral** genome.

CLMS(53)

53. The **adenoviral vector** of claim 52, wherein said another region is the E1 region of the **adenoviral** genome.

CLMS(54)

54. The **adenoviral vector** of claim 52, wherein said **adenoviral** genome is deleted of the E4 region of the **adenoviral** genome.

CLMS(55)

55. The **adenoviral vector** of claim 54, wherein said

**adenoviral** genome is deficient in one or more other essential gene functions of another region of the **adenoviral** genome.

CLMS(56)

56. The **adenoviral vector** of claim 52, wherein said **adenoviral** genome is deleted of the E4 region of the **adenoviral** genome except the E4 promoter and the E4 polyadenylation sequence.

CLMS(57)

57. The **adenoviral vector** of claim 56, wherein said **adenoviral** genome is deficient in one or more other essential gene functions of another region of the **adenoviral** genome.

CLMS(58)

58. The **adenoviral vector** of claim 49, wherein said **adenoviral** genome is deleted of the E4 region of the **adenoviral** genome except the E4 polyadenylation sequence.

CLMS(59)

59. The **adenoviral vector** of claim 50, wherein said **adenoviral** genome is deficient in one or more essential gene functions of another region of the **adenoviral** genome.

CLMS(60)

60. The **adenoviral vector** of claim 49, wherein said **adenoviral** genome is further deficient in the E2A region of the **adenoviral** genome.

CLMS(61)

61. The **adenoviral vector** of claim 60, wherein said E2A region comprises a portion of the **adenoviral** genome which encodes amino acids of the Nt domain of the DBP sufficient to allow for viral production, and wherein said E2A region is deficient in the portion of the **adenoviral** genome which encodes amino acid sequences of the Ct domain that are necessary for DNA binding.

CLMS(62)

62. A replication competent **adenovirus**-free stock of the **adenoviral vector** of any of claims 49-61.

CLMS(63)

63. A method of increasing the propagation in a complementing cell line of an **adenoviral vector** comprising an **adenoviral** genome deficient in the E4 region, which method comprises incorporating into the E4 region of the **adenoviral** genome of said **adenoviral vector** a spacer comprising at least about 15 base pairs, wherein said spacer comprises a passenger gene that is non-native to the E4 region of the **adenoviral** genome, thereby resulting in an increase in the viral production level of said **adenoviral vector**.

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CLAIMS:

CLMS(1)

We claim:

1. An **adenoviral vec** comprising an **adenovirus** geno from which one or more of the E4 open reading frames has bee deleted, but retaining sufficient E4 sequences to promote virus replication in vitro, and additionally comprising a DNA sequence of interest operably linked to expression control sequences and inserted into said **adenoviral** genome.

CLMS(2)

2. The **vector** of claim 1 wherein a PGK promoter is operably linked to the DNA sequence of interest.

CLMS(3)

3. The **vector** of claim 1 from which the Ela and Elb regions of the **adenovirus** genome have been deleted.

CLMS(4)

4. The **vector** of claim 1 from which the E3 region of the **adenovirus** genome has been deleted.

CLMS(5)

5. The **adenoviral vector** of claim 1 in which open reading frame 6 of the E4 region is retained in the **adenovirus** genome.

CLMS(6)

6. The **adenoviral vector** of claim 1 in which open reading frame 3 of the E4 region is retained in the **adenovirus** genome.

CLMS(7)

7. The **adenoviral vector** of claim 1 wherein the DNA sequence encodes cystic fibrosis transmembrane regulator protein.

CLMS(8)

8. The **adenoviral vector** of claim 2 wherein the DNA sequence encodes cystic fibrosis transmembrane regulator protein.

CLMS(9)

9. The **adenoviral vector** of claim 3 wherein the DNA sequence encodes cystic fibrosis transmembrane regulator protein.

CLMS(10)

10. The **adenoviral vector** of claim 3 wherein the DNA sequence is inserted into the deleted Ela and Elb regions of the **adenoviral** genome.

CLMS(11)

11. The **adenoviral vector** of claim 5 wherein the DNA sequence encodes cystic fibrosis transmembrane regulator protein.

CLMS(12)

12. The **adenoviral vector** of claim 6 wherein a cytomegalovirus promoter is operably linked to the DNA sequence of interest.

CLMS(13)

13. A method for providing cystic fibrosis transmembrane conductance regulator protein to airway epithelial cells of a cystic fibrosis patient comprising administering directly to airway epithelial cells of the patient an **adenoviral vector**, said **vector** comprising an **adenovirus** genome from which one or more E4 open reading frames has been deleted, but retaining sufficient E4 sequences to promote virus replication in vitro, and additionally comprising a DNA sequence encoding cystic fibrosis transmembrane regulator protein operably linked to expression control sequences and inserted into the E1 region said **adenoviral** genome, under conditions whereby the DNA sequence encoding cystic fibrosis transmembrane regulator protein is expressed and a functional chloride ion channel is produced in the airway epithelial cells of the patient.

CLMS(14)

14. The method of claim 13 wherein open reading frame 6 of the E4 region of the **adenovirus** genome is retained in the **vector**.

CLMS(15)

15. The method of claim 13 wherein the expression control sequences operably linked to the DNA sequence comprise the PGK promoter.

CLMS(16)

16. The method of claim 13 in which the Ela and Elb regions of the **adenovirus** genome of the **vector** have been deleted.

CLMS(17)

17. The method of claim 13 in which the E3 region of the **adenovirus** genome of the **vector** has been deleted.

CLMS(18)

18. The method of claim 13 wherein open reading frame 3 of the E4 region of the **adenovirus** genome is retained in the **vector**.

CLMS(19)

19. The method of claim 18 wherein the expression control sequences operably linked to the DNA sequence comprise a cytomegalovirus promoter.